

Besides control groups previously treated or not with AngII ( $10^{-10}$  M for 50 minutes), the experimental groups – cells overexpressing one of the recombinant proteins LacZ/myc-His, LacZ-V5-His, AT1R-myc-His and ATRAP-V5-His – were also treated with AngII. Our results showed, as expected, a significant faster pHi recovery rate in OKP cells treated with AngII ( $6,544 \pm 0,8425$  min in untransfected cells;  $3,059 \pm 0,4659$  min in untransfected cells with AngII;  $2,784 \pm 0,6104$  and  $2,641 \pm 0,3911$  min in LacZ/myc-His and LacZ/V5-His transfected cells with AngII, respectively). Despite our initial hypothesis, we did not observe a faster pHi recovery rate in OKP cells overexpressing AT1R-myc-His ( $3,026 \pm 0,5445$  min), what could be due to lack of expression of functional recombinant proteins, since the protein size on Western blot was lower than expected, or consequence of redundant effect on intracellular signaling. On the other hand, ATRAP overexpression decreased pHi recovery rate in comparison with the other experimental groups treated with AngII ( $4,423 \pm 0,9867$  min). Our results support the hypothesis that ATRAP plays an inhibitory effect on Ang/AT1R modulation of NHE3.

### 1315-Pos Board B266

#### Environmental Factors Allowing Stem Cells from Skeletal Muscle Turning into Cardiac Muscle Like Spontaneous Beating Cells

Hikari Hayashida, Wataru Yamasaki, Takayuki Miyanishi.

Department of Environmental Studies, Nagasaki University, Nagasaki, Japan.

Since skeletal muscle-based precursor of cardiomyocytes (SPOC), a stem cell that potentially differentiates into heart cells, was reported for leg skeletal muscle of mouse [Winitsky et al. (2005) PLoS Biol 3(4), e87], we have studied the environmental conditions, type of muscles, preparation methods which may allow us to estimate origin of cells and factors to make cells consistently turning into spontaneous beating cells. In this paper, whether or not satellite cells are their origin, either which exogenous factors or which pathway of signal transduction are required for differentiation to spontaneous beating cells. Medium condition and extracellular matrix to induce cells into spontaneous beating cells appeared to be different between whole muscle cells and satellite cells.

### 1316-Pos Board B267

#### Roles of Phosphodiesterases in Cyclic Nucleotide Cross-Talk in Cardiac Myocytes

Claire Y. Zhao, Joseph L. Greenstein, Raimond L. Winslow.

Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA.

The cyclic nucleotides (cNs) cAMP and cGMP regulate the response of cardiac myocytes to both external and internal stimuli. Distinctly-regulated phosphodiesterases (PDEs) control the degradation of these cNs. As a result of their regulation by cNs, PDEs also facilitate communication between the  $\beta$ -adrenergic and Nitric Oxide (NO)/cGMP/Protein Kinase G (PKG) signaling pathways, which regulate the synthesis of cAMP and cGMP, respectively. Activation of the  $\beta$ -adrenergic pathway potentiates cardiac contractility, whereas activation of the NO/cGMP/PKG pathway reduces it. As a result, the balance between cNs plays a critical role in regulating contraction. The phenomena where cAMP influences the dynamics of the cGMP pathway, and vice versa, are commonly referred to as cN cross-talk. However, the cross-talk response and the individual role of each PDE isoenzyme in shaping this response remain to be fully characterized. We have developed a computational model of the cN cross-talk network that mechanistically integrates the  $\beta$ -adrenergic and NO/cGMP/PKG pathways via regulation of PDEs by both cNs. The individual model components and the entire network model replicate experimentally observed activation-response relationships and temporal dynamics. The model predicts that under sub-maximal  $\beta$ -adrenergic stimulation, an increase of PDE2 and a decrease of PDE3 cAMP hydrolysis rates under concomitant NO stimulation results in a net cAMP accumulation, leading to the observed NO-mediated potentiation of Protein Kinase A (PKA) activation. In addition, under concomitant  $\beta$ -adrenergic stimulation, due to cGMP accumulation from increased PDE5 and decreased PDE3 cGMP hydrolysis rates, PKG can be further activated beyond the level achieved under NO alone. By defining cN cross-talk reactions based on the binding affinity of cNs to specific PDE domains and the associated hydrolysis rates, we pin-pointed the principal mechanisms driving the cross-talk response within this non-linear, tightly-coupled reaction system.

### 1317-Pos Board B268

#### Regulation of the Cardiac $\beta$ -Adrenergic Pathway via Camp-Cgmp Competition

Claire Y. Zhao, Joseph L. Greenstein, Raimond L. Winslow.

Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA.

In the cardiac myocyte, the second messenger cAMP is synthesized by the  $\beta$ -adrenergic signaling pathway upon sympathetic activation. It activates Protein Kinase A (PKA) mediated phosphorylation of downstream targets that are critical to the control of cardiac contractility. The dynamics of cAMP are also controlled indirectly by cGMP-mediated regulation of phosphodiesterase (PDE) isoenzymes. It is not yet clear how the cGMP signal is transduced by the PDEs to regulate cAMP. To better understand this, we have developed mechanistically detailed models of PDEs 1 - 4, the primary cAMP hydrolyzing PDEs in cardiac myocytes, and integrated them into an existing model of the  $\beta$ -adrenergic signaling pathway. Our PDE models are based upon experimental studies performed on purified PDE enzymes which show that cyclic nucleotides (cNs) bind competitively to the domains of PDEs 1, 2, and 3. PDE4 is regulated by PKA but does not interact appreciably with cGMP. Our individual PDE models reproduce cAMP hydrolysis rates as regulated by various cGMP concentrations, and the fully integrated model also replicates experimentally observed cAMP dose-response relationships and temporal dynamics. Our model shows that PDE2 is critical to the regulation of cAMP signals, especially during increased stimulation of the  $\beta$ -adrenergic pathway. In addition, it reveals that high levels of cGMP out-compete cAMP for catalytic sites of PDEs 1, 2, and 3, and suppress their cAMP hydrolysis rates. This leads to a net accumulation of cAMP despite the negative feedback of the cAMP-driven increases in rate of PDE4-mediated cAMP hydrolysis. These results provide insights into how PDEs serve as an integration point for cN signals and how cN interactions regulate  $\beta$ -adrenergic response.

### 1318-Pos Board B269

#### Intracellular Signaling Pathway of Cardiac Apoptosis in the Prediabetic Heart

Leandro Sommese<sup>1</sup>, Marilen Federico<sup>2</sup>, Carolina Zanuzzi<sup>3</sup>,

Enrique L. Portiansky<sup>3</sup>, John Dedman<sup>4</sup>, Marcia Kaetzel<sup>4</sup>,

Wehrens H. Xander<sup>5</sup>, Alicia Mattiazzi<sup>1</sup>, Julieta Palomeque<sup>6</sup>.

<sup>1</sup>Centro de Investigaciones Cardiovasculares, Fac. de Cs. Médicas; UNLP, CONICET-CCT La Plata, La Plata, Argentina, <sup>2</sup>Centro de Investigaciones Cardiovasculares, Fac. de Cs. Médicas; UNLP, CONICET-CCT La Plata, La Plata, Argentina, <sup>3</sup>Fac. de Cs. Veterinarias. UNLP, CONICET-CCT La Plata, Argentina, La Plata, Argentina, <sup>4</sup>Department of Genome Science, University of Cincinnati College of Medicine, Cincinnati, OH, USA, <sup>5</sup>Cardiovascular Research Institute, Department of Molecular Physiology and Biophysics, Department of Medicine (in Cardiology), Baylor College of Medicine, Houston, TX, USA, <sup>6</sup>Centro de Investigaciones Cardiovasculares, Fac. de Cs. Médicas; UNLP, CONICET-CCT La Plata, La Plata, Argentina.

Apoptosis leads cardiac dysfunction and heart failure, which are more frequently in people with diabetes than in the general population. However, in impaired glucose tolerance (IGT), which characterizes prediabetic state, apoptosis has not been evaluated in heart. Moreover, although CaMKII is related with cardiac apoptosis, the connection with IGT is unknown. Thus, the present study aimed to evaluate apoptosis in IGT heart and its putative link with CaMKII activity.

IGT was induced by a fructose-rich diet (control, CD; and fructose, FRD; rats or mice). Echocardiography, biochemical studies, reactive oxygen species (ROS),  $\text{Ca}^{2+}$  measurements, mitochondrial swelling and mitochondria membrane potential measurements were performed.

FRD rats showed decreased contractility and increased hypertrophy (echocardiography) associated with increased CaMKII activity (P-CaMKII  $191.6 \pm 18.3$ ), and ROS ( $185.4 \pm 28.6\%$ ) vs CD rats (100%). TUNEL positive nuclei and Bax/Bcl2 ratio was increased in FRD vs CD rats ( $273.6 \pm 39.7\%$ ). Mitochondria from FRD rats showed significant more swelling ( $\Delta\text{DFO } 0.34 \pm 0.05$  CD vs  $0.53 \pm 0.03$  FRD) and enhanced membrane depolarization than CD mitochondria. Myocytes from FRD rats showed a significant increase in sarcoplasmic reticulum (SR)  $\text{Ca}^{2+}$  leak vs CD myocytes. FRD SR-AIP mice (which express the CaMKII inhibitory peptide [AIP] at the SR membranes) showed less TUNEL positive nuclei than their matched FRD control mice. FRD control mice co-treated with the ROS scavenger, tempol, showed less apoptosis than the one induced by fructose alone. SR  $\text{Ca}^{2+}$  leak was also prevented in either FRD SR-AIP mice or CD mice co-treated with tempol. Mitochondria swelling was also prevented in S2814A mice, which ryanodine receptor (RyR2) cannot be phosphorylated by CaMKII.

The results would indicate that the signaling apoptotic cascade in IGT hearts involves mitochondria damage by SR  $\text{Ca}^{2+}$  leak produced by CaMKII-dependent phosphorylation of RyR2. CaMKII would be activated by both,  $\text{Ca}^{2+}$  and ROS.